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Article type: Research Article

Handling Editor: Adam Martin

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Functional traits in cover crop mixtures: biological nitrogen fixation and multifunctionality

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ABSTRACT

1. Cover crop mixtures with complementary plant functional traits including biological nitrogen fixation (BNF) may supply nitrogen (N) to farm fields while simultaneously providing other ecosystem functions such as N retention and weed suppression (i.e., multifunctionality). Understanding variation in these relationships across farms can help advance trait-based research in agroecology and ecological approaches to nutrient management.

2. This on-farm experiment explored the contributions of two- and three-species cover crop mixtures, which combined legumes, brassicas, and cool season grasses, to ecosystem functions across a gradient of soil fertility levels driven by farm management history.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1365-2664.13011](https://doi.org/10.1111/1365-2664.13011)

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3. I evaluated the predictions that functional trait diversity of the cover crops would explain variation in multifunctionality, and that legume biomass and BNF within mixtures would be inversely correlated with indicators of soil N availability from organic matter across the farm gradient.
4. Ecosystem functions varied widely across farms. As expected, functional diversity was a significant predictor of multifunctionality, although the relationship was weak. Cover crop mixtures had significantly greater multifunctionality than a cereal rye monoculture, though not at the highest observed levels of each function, indicating trade-offs among functions. Linear regression models showed that legume biomass and BNF were negatively correlated with soil properties indicative of N availability from soil organic matter, whereas non-legume and weed biomass were positively correlated with measures of soil fertility.
5. *Synthesis and applications.* Cover crop mixtures can increase functional diversity within crop rotations. Designing mixtures with complementary plant traits may be particularly effective for increasing multifunctionality and agroecosystem sustainability. On-farm research to understand variation in biological nitrogen fixation (BNF), which is both a plant trait and a key ecosystem function, across heterogeneous soil conditions, can inform management of soil fertility based on ecological principles.

Keywords: agroecology, biological nitrogen fixation, cover crops, ecological nutrient management, functional diversity, functional trait, multifunctionality, on-farm research, plant traits, soil fertility

INTRODUCTION

In agroecosystems, small increases in biodiversity can lead to large benefits for ecosystem function (Jackson et al., 2007, Drinkwater et al., 1998, Tiemann et al., 2015). The specific impacts of this “intended” biodiversity on agroecosystem processes can be evaluated based on species richness, other taxonomic diversity metrics, or more recently, plant functional traits (Petchey and Gaston, 2006, Wood et al., 2015, Garnier and Navas, 2012, Martin and Isaac, 2015). By managing functional trait diversity, farmers manipulate ecological interactions such as competition or facilitation to support ecosystem functions including nutrient supply, nutrient retention, weed and pest suppression, and organic matter accrual (Shennan, 2008).

Cover crop mixtures can increase the functional trait diversity of crop rotations during windows between cultivation of primary crops. Increasingly, a wide range of farmers express interest in planting multi-species cover crop mixtures to enhance ecosystem functions (CTIC et al., 2016). To date, much of the empirical research on biodiversity and ecosystem function has focused on single functions, but there is growing interest in understanding the relationship between diversity and *multifunctionality*, which is defined as the simultaneous enhancement of multiple ecosystem functions (Byrnes et al., 2014). Recent research in natural ecosystems indicates that when considering multiple ecosystem functions together, increasing species richness may augment complementary functions (Zavaleta et al., 2010, Mori et al., 2016).

Within agroecosystems, however, studies on cover crop mixtures have not found strong relationships between species diversity and multiple ecosystem functions even though the mixtures over-yielded compared to monocultures (Wortman et al., 2012, Smith et al., 2014). Finney and Kaye (2017) found that increasing cover crop species richness of an agroecosystem only weakly correlated with multifunctionality. Instead, metrics of functional diversity based on plant functional traits of the cover crop mixtures – fall and spring growth rates and shoot C:N – better predicted multifunctionality in their field experiment. Similarly, Storkey et al. (2015) reported that cover crops of one- to four-species, which represented contrasts in functional traits such as biological N fixation (BNF) and phenology, enhanced the provisioning of ecosystem services compared to higher diversity mixtures. Taken together, these studies suggest that cover crop mixtures that combine complementary plant functional traits may optimize agroecosystem functions.

Symbiotic dinitrogen (N_2) fixation by legume species is a particularly valuable plant trait in agroecosystems. Crop rotations with BNF as the primary N source can have low or no N surpluses; that is, field-scale N inputs and harvested N exports are approximately in balance (Zhang et al., 2015, Blesh and Drinkwater, 2013). Legumes may down-regulate BNF and increase their dependence on soil N as soil organic N pools increase because of the energetic cost of supplying C to their symbiotic partners (Kiers et al., 2003). Further, overwintering legume cover crops reduce nitrate leaching because winter cover extends the timeframe of plant N uptake (Tonitto et al., 2006). Legumes can also contribute to long-term accumulation of soil organic matter (SOM) (e.g., Drinkwater et al. 1998).

Although BNF is often characterized as a binary functional category (Brooker et al., 2015) –

presence or absence of legumes – the rate of N fixation (% N from fixation) varies within and across legume species. BNF can therefore be considered both a continuous plant trait *and* a critical ecosystem function that provides a new input of fixed N. The N fixation rate varies with competitive interactions in mixtures; for example, in legume-grass mixtures the legume's reliance on BNF increases due to competition for soil N by the grass species (e.g., Jensen, 1996). Legume N fixation is also likely to vary with soil fertility and management history. For instance, the outcome of competitive and facilitative interactions between legumes and non-legumes in mixtures may vary with soil fertility status and N supply from SOM (Schipanski and Drinkwater, 2011). However, as long as there are effective rhizobia in the soil, the N supply from BNF will largely be governed by total legume biomass production rather than by the % of legume N from fixation (Schipanski and Drinkwater 2011, Crews et al. 2016).

This study integrates functional ecology and ecological nutrient management frameworks to assess how soil fertility status affects ecosystem functions from cover crop mixtures across working farms. The specific objectives are to: i) test relationships between functional trait diversity of cover crop mixtures and multifunctionality; and, ii) identify soil characteristics that explain variation in BNF in cover crop mixtures across farms. I evaluated 9 cover crop treatments with 1, 2, or 3-species, along with a no-cover control, on 8 organic vegetable farms in southeastern Michigan. Treatments harnessed contrasts in several continuous and complementary plant traits: BNF, fall and spring growth rates, and shoot C:N ratio. I used three ecosystem functions to assess multifunctionality: N supply from BNF, weed suppression during the cover crop season, and N retention in aboveground biomass. I expected monocultures to maximize individual functions compared to mixtures, and functional diversity of the treatments to predict multifunctionality. I also predicted that legume biomass and BNF would be inversely correlated with measures of N availability from SOM, and that plant-available phosphorus (P) would correspond with greater legume and weed biomass.

MATERIALS AND METHODS

Experimental design

In the winter and spring of 2014, I recruited 8 vegetable farmers in southeastern Michigan who manage their farms organically to investigate how cover crop mixtures combining diverse functional traits impact ecosystem functions. Farms had been in organic vegetable production

from 1 to 13 years, and fields represented a gradient in soil fertility due to management history. Six of the cover crop treatments were mixtures that included a legume and a grass species. The mixtures combined winter- and non-winter-hardy species, except for one treatment with three species that winter-kill (LN+YM+OA)¹. As a result, mixtures represented combinations of complementary plant functional traits: fall growth potential (kg ha^{-1} growing degree day (gdd^{-1})), spring growth potential (kg ha^{-1} gdd^{-1}), C:N ratio of plant shoots, and BNF. The study also had three single-species treatments –including cereal rye (CR), which is the most common cover crop grown in the region and thus a useful benchmark for comparison – as well as a no cover crop control (Table S1, Appendix S1, Supporting Information).

All experimental treatments were established on farms between August 13 - 20, 2015, in a randomized complete block design with four replicates. Each plot was 2.4 x 2.4m (5.95m²). Legume seeds were inoculated with the appropriate inoculant (Nitragin® Gold or N-Dure®) at a rate of approximately 4 g kg⁻¹ seed.

Soil sampling and analysis

Soil samples for baseline characterization of soil properties and metrics of soil nutrient cycling capacity were collected before establishment of the experiment from approximately 20 soil cores (2 cm diameter by 20cm depth), composited per experimental field to represent the initial conditions of each site. Since these were diversified vegetable farms, fields were relatively small (283 – 590 m², or 0.03-0.06 ha), flat, and homogeneous. I measured bulk density from the fresh weight of 8 cores per field using a field scale, and adjusted for soil moisture. Soil was processed immediately for soil moisture and extractable inorganic N (NO_3^- and NH_4^+). Triplicate soil subsamples were sieved for inorganic N determination and for a 7-day anaerobic N mineralization incubation (Drinkwater et al., 1996) followed by extraction with 2 M KCl. The amount of NH_4^+ and NO_3^- in each sample was analyzed colorimetrically on a continuous flow analyzer (AQ2, Seal Analytical, Mequon, WI). Remaining soil was air-dried before further analysis.

¹ List of treatments and abbreviations (see also, Table S1): 1) Crimson clover, Medium red clover, and spring wheat (CC+RC+SW); 2) Austrian winter pea, oat, and daikon radish (WP+OA+DR); 3) Lentil, yellow mustard, and oat (LN+YM+OA); 4) Medium red clover and spring wheat (RC+SW); 5) Crimson clover and spring wheat (CC+SW); 6) Chickling vetch and cereal rye (CV+CR); 7) Austrian winter pea (WP); 8) Cereal rye (CR); 9) Spring wheat (SW); and 10) weedy fallow control.

SOM has different fractions representing a continuum of accessibility to microbial decomposition, which therefore supply N over different timescales. Soil particulate organic matter (POM) fractions, in particular, respond to changes in management on shorter timescales (years to decade), and are indicators of soil nutrient supplying capacity relevant for guiding farm management decisions (Wander, 2004). Light fraction particulate organic matter (POM; also called free POM, or fPOM), and occluded POM (oPOM; i.e., physically protected POM), were separated on triplicate 40 g subsamples using a size and density fractionation method (Marriott and Wander, 2006, Appendix S2). Total soil C and N (to 20cm) were measured by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI), and the C and N content of fPOM and oPOM were measured on a Costech ECS 4010 CHNS Analyzer (Costech Analytical, Valencia, CA). A subset of approximately 100g of sieved dried soil was analyzed for particle size (texture), pH, Bray-1 P, K, and other macro- and micro-nutrients at A & L Great Lakes Laboratories, Inc. (Fort Wayne, IN).

Aboveground biomass sampling and C and N analysis

Aboveground biomass in all treatments was sampled in the fall between 5 and 22 October, 2015, and in the spring between 26 April and 18 May 2016 from one random 0.25 m² section of each replicate plot avoiding plot edges. Biomass was cut at the soil surface, separated by species (weeds were combined into one pool), dried at 60 °C for 48 h, weighed, and ground in a Wiley mill. Shoot biomass was analyzed for total C and N by dry combustion on a Leco TruMac Analyzer. Samples for isotope analysis were pulverized using a cyclone mill and analyzed at the UC Davis Stable Isotope Facility (see *Legume N fixation*).

Legume N fixation

I estimated BNF using the natural abundance method (Shearer and Kohl, 1986). Briefly, legume and reference plant biomass (from CR and SW monocultures) were analyzed for ¹⁵N enrichment and total N content using a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis).

The %N derived from fixation was calculated using the following mixing model:

$$\%N \text{ from fixation} = 100 * ((\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{legume}}) / (\delta^{15}N_{\text{ref}} - B))$$

where $\delta^{15}N_{\text{ref}}$ is the $\delta^{15}N$ signature of the reference plant, $\delta^{15}N_{\text{legume}}$ is the $\delta^{15}N$ signature of the legume, and B is defined as the $\delta^{15}N$ signature of a legume when dependent solely on atmospheric N_2 . B values were determined by growing each legume species in the greenhouse in a N-free medium (Appendix S2).

Calculation of functional diversity

To link ecosystem functions to functional diversity, I calculated Rao's Quadratic Entropy (Rao) (Rao, 1982, Schleuter et al., 2010) for each treatment using *FDiversity* software (Di Rienzo et al., 2008) (Appendix S3). I used the total aboveground biomass at the fall sampling date to weight the index by abundance, since at that time all species, including the non-overwintering species, were represented in the plots (i.e., some of the mixture treatments became monocultures following winterkill). The functional diversity index included four continuous plant functional traits: fall growth potential ($\text{kg ha}^{-1} \text{ gdd}^{-1}$), spring growth potential ($\text{kg ha}^{-1} \text{ gdd}^{-1}$), C:N ratio of plant shoots, and proportion of legume shoot N from fixation. To avoid scale effects, trait values were standardized to have zero mean and unit variance.

Calculation of multifunctionality

Four ecosystem functions were measured: total aboveground biomass production, N retention in aboveground biomass, N supply from BNF, and weed suppression (Appendix S3). Following Byrnes et al. (2014), I calculated a threshold-based index of multifunctionality, selecting three threshold levels potentially relevant to management (Table 1). Total aboveground biomass correlated with weed suppression, N retention, and BNF (see *Results*). I therefore did not include biomass in the calculation of multifunctionality, and the maximum score in the index was 3. I calculated multifunctionality at three different threshold levels (30%, 50%, and 75%) of the maximum observed level of each function, where the maximum value was the mean of the top 10 observations for each function across farms (Table 1). For example, a treatment would receive a multifunctionality score of 3 at the 30% threshold if BNF input was greater than 46 kg N ha^{-1} , and weed suppression was greater than $1705 \text{ kg dm ha}^{-1}$, and soil N retained in biomass was greater than 59 kg ha^{-1} . I applied a square root transformation to the data for the three functions

prior to calculating the maximum value since the distributions were skewed.

Statistical analysis

All statistical analyses were computed in R (The R Foundation for Statistical Consulting, Vienna, Austria) using the *lme4* package for linear, mixed-effect models with treatment as a fixed effect and block nested in farm as a random effect. Comparison of least square means was performed using Tukey's honestly significant difference (HSD). Results are reported as statistically significant at $\alpha = 0.05$.

Since legume biomass governs the N supply from BNF (e.g., Crews et al., 2016), as well as the abundance of the N fixation trait within cover crop mixtures, I used linear regression to model aboveground biomass for each species in each treatment as a function of soil properties. I first selected a subset of soil predictors using information from the correlation matrix of all soil parameters (due to multicollinearity among soil variables and small sample size), and specific hypotheses about parameters that may drive variation across farms. Model selection was also informed by model comparisons to assess goodness of fit with the Akaike Information Criterion (AIC). In addition to legume biomass, I also modeled the BNF trait – both aboveground N from fixation and % of shoot N from fixation (and % from soil) – for the legume species. In addition to the soil predictors, I also included legume and weed biomass in these regressions, and then dropped weed biomass for most of the models, which had better fits without this predictor.

RESULTS

Baseline soil properties

Soil analyses from farm fields indicated a gradient of soil fertility that reflected different farm management histories as well as underlying soil type (Table S2). All soils were Alfisols or Mollisols. Bray-1 P concentrations ranged from 4 - 88 ppm. Total organic C varied twofold from 27 - 52 Mg ha⁻¹. Potentially mineralizable N, fPOM pool size, and the quality of fPOM and oPOM pools (C:N) reflect soil N availability from more recent management practices and organic matter inputs (Wander 2004); fPOM pool size varied from 8.6 - 27.1 Mg ha⁻¹ and the N content of the oPOM pool ranged from 90.4 - 231.2 kg ha⁻¹ (Table S2).

Ecosystem functions during the cover crop season

Ecosystem functions for all treatments are shown in Figure 1. Of the monocultures, CR provided the greatest biomass production, weed suppression (relative to the no cover crop control), and N retention. Weed biomass was high, averaging $3981.9 \pm 352.9 \text{ kg ha}^{-1}$ for the cover crop season. CR biomass was more than twofold greater than SW and WP monoculture biomass, and biomass in SW, WP, RC+SW and LN+YM+OA treatments was lower than weed biomass in the control. CV+CR mixture biomass was not significantly different from CR, and several mixtures were not significantly different from CR in terms of weed suppression (CV+CR, LN+YM+OA, CC+SW) and N retention (CV+CR, LN+YM+OA). Mean fixed N inputs were lowest in CV+CR (fall BNF only; $13.5 \text{ kg N ha}^{-1}$). For the treatments with overwintering legumes, mean BNF ranged from 33.4 (RC+SW) to 59.0 (CC+RC+SW) kg N ha^{-1} (Figure 1, top panel). Soil N retention in plant biomass varied from 30.8 - 101.9 kg N ha^{-1} (in WP and CR, respectively), and total aboveground N accumulation (soil plus fixed N) ranged from 54.0 - 118.9 kg N ha^{-1} (in SW and CC+CR+SW, respectively).

Ecosystem functions varied widely across farms, in part driven by differences in cover crop biomass (Figures 2 and 3). For soil N retained in cover crop biomass (Figure 2, top), treatments without legumes had a greater amount of soil-derived N per unit biomass than did the treatments with legume species, and for both plant types this relationship was relatively strong (non-legume $R^2=0.69$, and legume $R^2=0.42$). The relationship between biomass and weed suppression (Figure 2, bottom) was weaker, though significant ($R^2 = 0.28$ for non-legumes, and 0.17 for legumes; $P<0.0001$), but the treatments with legume species had more variable weed suppression per unit cover crop biomass than did treatments with non-legumes only.

Across farms and legume species, the N supplied from BNF varied from 7 - 268 kg N ha^{-1} (Figure 3). There was a strong relationship between legume aboveground biomass and N from fixation (Figure 3; $R^2 = 0.95, 0.89, \text{ and } 0.82$ for WP, CC and RC combined, and CV, respectively). The slope of this relationship was greatest for WP. For the mixtures, there was also a positive relationship between legume biomass as a proportion of total mixture biomass and N supply from BNF (data not shown).

Multifunctionality

The CV+CR treatment had the greatest functional diversity score (Rao), followed by all other treatments with legumes, with the exception of LN+YM+OA in which the lentil (LN) performed poorly (Table 2). There was a significant relationship across treatments and farms between cover crop functional diversity and multifunctionality, but functional diversity only explained a small portion of the variation in multifunctionality (Figure 4a; $P=0.0003$; $R^2=0.05$). At the 30% threshold, the CC+RC+SW mixture had the greatest mean multifunctionality index (Figure 4b and Table 2; 2.5). This score was not significantly different from WP+OA+DR (2.3), CC+SW (2.4), or the WP monoculture (2.2). The control (no cover) had the lowest level of multifunctionality at the 30% level. For all treatments, the mean number of ecosystem functions provided decreased as the threshold increased (Figure 4b). Comparing the mixtures to the CR monoculture, three mixtures had a score significantly greater than CR at the 30% threshold. At 50%, their scores started to overlap with CR, and at the 75% level, all of the multifunctionality scores were low, and were not different from CR (Table 2). There was a significant, but weak relationship between biomass and multifunctionality (Figure S1; $P<0.0001$; $R^2=0.14$) for observations from all treatments and farms.

Soil characteristics as predictors of BNF across farms

To understand drivers of variation in the N fixation trait across farms, regression using soil properties to model biomass across the 8 farm fields was separated into biomass for legume species (Table 3), non-legumes (grasses and brassicas, Table 4), and weeds (Table 5) for each treatment. Model selection identified soil properties to include in the regression models that: i) were not strongly correlated with one another, and, ii) tested hypotheses about SOM fractions that are responsive to management and have faster turnover times than the total SOM pool. As expected, biomass for some legume species was negatively correlated with soil properties indicative of soil N cycling capacity (Table 3): RC was positively related to the C:N of the fPOM (i.e., higher C:N reflects fPOM of lower N fertility), and CC and WP biomass were negatively correlated with the size of the fPOM pool (i.e., quantity of fPOM). Both CC and WP biomass were negatively related to the oPOM N pool. WP biomass in both monoculture and mixture was positively correlated with plant-available P, but this coefficient was not significant for other legume species. None of the models were significant for predicting RC biomass across the farms, and the model for CV had a low R^2 (0.31). Models for the other legume species were strong

(Table 3, $R^2 = 0.60 - 0.79$), particularly considering the relatively small number of sites and high variation typical of environmental data.

The models with the greatest fit for the non-legumes (Table 4) were for SW biomass in CC+SW ($R^2=0.52$), SW biomass in RC+SW ($R^2=0.63$) and CR biomass in CV+CR ($R^2=0.58$). Non-legume biomass was positively correlated with higher soil fertility; i.e., larger fPOM pool size, higher concentration of plant-available P, and higher % clay (for CR), which is often correlated with total SOM. Models for weed biomass within the cover crop treatments (Table 5) were weaker ($R^2 = 0.20 - 0.37$), with the highest R^2 for the model of weed biomass in the no cover crop control ($R^2=0.46$). Weed biomass was also positively correlated with indicators of soil fertility including % clay, a narrower C:N (i.e., a negative relationship with the C:N of the fPOM), soil P concentration (for weeds in LN+YM+OA and in the control), and with the size of the fPOM pool (for weeds in control).

Legume biomass was the strongest predictor in the models of BNF (i.e., shoot N from fixation in kg N ha^{-1} ; $P < 0.0001$ for all species; Figure 3). Total shoot N fixed by WP in WP+OA+DR and RC in RC+SW was negatively correlated with weed biomass ($P=0.007$ and 0.046 , respectively); these two mixtures also had the lowest weed suppression (Figure 1, middle). BNF (kg N ha^{-1}) by CC in CC+RC+SW and CC+SW was negatively correlated with % clay. RC BNF was positively correlated with soil P for both RC treatments ($P=0.03$ and 0.04), and CC and RC % N from soil was inversely related to soil P concentration ($P=0.008$ for CC in CC+RC+SW; $P=0.0001$ for all others). Models of % legume N from soil were also positively correlated with total legume biomass. In contrast to legume biomass and aboveground N from fixation, soil properties did not predict the % of aboveground N from fixation for any species, although CC % N from fixation in the spring (in both treatments) was positively related to increasing C:N of the fPOM pool (i.e., lower N availability). Models for legume biomass as a proportion of total mixture biomass had lower predictive power than models for legume biomass itself, but showed similar correlations with soil properties.

DISCUSSION

Functional trait diversity can provide multiple benefits in agroecosystems (Martin and Isaac, 2015). For example, cover crop mixtures that include legumes can supply N while simultaneously providing other ecosystem functions (e.g., Schipanski et al., 2014). An emerging

ecological framework for nutrient management has demonstrated increased N use efficiency in rotations with legume N sources, winter cover crops, and/or perennials (Gregorich et al., 2001, Drinkwater et al., 1998, Blesh and Drinkwater, 2013). Since winter cover crops can increase functional diversity without requiring major changes to crop rotations, the practice is applicable to a broad range of farms.

Building on evidence suggesting that functional diversity in cover crop mixtures predicts multifunctionality (Finney and Kaye, 2017), I tested the hypothesis that cover crop mixtures selected to leverage contrasts in plant traits – shoot N concentration, timing of peak growth, and BNF – would provide greater multifunctionality compared to cover crop monocultures and a no-cover control across farms in southeastern Michigan. Soil types on the farms were all Alfisols and Mollisols, but fields varied in metrics of soil nutrient cycling capacity that reflected differences in management history and underlying soil texture. I therefore also tested the hypothesis that soil N availability from SOM pools would explain variation in legume biomass and BNF across farms. Understanding how abiotic conditions drive variation in functional trait expression and cover crop performance is a critical research gap that can inform management based on principles of functional ecology.

Functional diversity of cover crop mixtures and ecosystem functions

Ecosystem functions varied widely along the farm gradient. The CR monoculture was the top performer for all individual ecosystem functions except for N supply from BNF, since it is a non-N-fixing species. CR is currently the most common winter cover crop grown in the region due to reliable establishment in the late fall after crop harvest and lower seed costs compared to legume species (Snapp et al., 2005). However, several mixtures were not significantly different from CR in terms of biomass production, N retention, and weed suppression, indicating opportunities for multifunctionality from mixtures that include legumes.

Across treatments and farms, cover crop biomass was positively correlated with other ecosystem functions (Figures 2, 3, and S1). The relationships for N retention and weed suppression were only slightly weaker than similar relationships reported by studies conducted at a single research site (e.g., Finney et al., 2016). The larger scatter in the relationships for the cover crop treatments with legumes, compared to treatments with non-legumes only, is the result of greater variability in legume biomass compared to non-legume biomass.

The relationship between legume biomass and fixed N input was very strong (Figure 3), which corresponds with other studies (e.g., Unkovich et al., 2010). The different slopes for different legumes indicated that WP was fixing N at the highest rate compared to other species, regardless of plant size. However, since legume biomass is a more important driver of total N supply than is % N from fixation (Crews et al., 2016), competitive interactions in mixtures may decrease fixed N inputs to agroecosystems if legume biomass is reduced.

Multifunctionality

The relationship between the functional diversity index (Rao) of the treatments and multifunctionality was significant across farms, but was weaker than that reported in a study conducted at one experimental site (Finney and Kaye, 2017). This difference in findings may be due to the greater variation across multiple farm sites, the smaller number of species tested in the mixture treatments in this study, or perhaps because this experiment included several cover crops that have been less commonly studied and did not perform well on the farms. Expression of particular plant functional traits depends on the successful establishment and growth of different species in mixtures (e.g., the biomass-ratio hypothesis, Grime, 1998); however, cover crop mixtures are still rare on working farms, and their management has not been optimized for a broad range of conditions.

Here, I assessed multifunctionality at three thresholds (i.e., percentages of the maximum observed level of each function), which is preferable to using a single threshold value since the outcomes depend on the threshold chosen (Byrnes et al., 2014). Results supported the hypothesis that mixtures would simultaneously enhance more ecosystem functions than the CR monoculture; however, the difference was only statistically significant at the 30% threshold. For all treatments, the mean number of ecosystem functions provided decreased with increasing thresholds, indicating that there are trade-offs limiting the ability of cover crop mixtures to provide multiple functions at high levels (Finney and Kaye, 2017, Schipanski et al., 2014). Table 1 translates the thresholds into absolute values for each function. Both the 30 and 50% thresholds provided substantial, management-relevant N input rates (46 - 77 kg N ha⁻¹ yr⁻¹), soil N retention in aboveground biomass (59 - 98 kg N ha⁻¹), and weed suppression (1705 - 2842 kg ha⁻¹ of weed dry matter suppressed compared to a no cover crop control). One drawback of this approach is that it does not identify whether each function passes a threshold by a small or large

amount (Byrnes et al., 2014). Assessment approaches like this one could be further developed together with farmers, to define relevant thresholds and manage functional diversity based on different goals.

Previous studies have explored legume-grass intercrops for simultaneously supplying and retaining N within agroecosystems (e.g., Ranells and Wagger, 1997, White et al., 2017). Mixtures of legumes and non-legumes commonly result in facilitation. For example, some of the N fixed by the legume can be directly transferred to the intercropped species through common mycorrhizal networks, or may indirectly increase N uptake by the non-legume via root exudation (and potentially priming effects), or root turnover (Munroe and Isaac, 2014, Høgh-Jensen and Schjoerring, 2001). Including estimates of fixed N transferred to intercropped species in the mixtures would likely increase their multifunctionality scores. Belowground N inputs are another area of uncertainty in estimating BNF inputs, and the aboveground N inputs reported here are thus underestimates (Høgh-Jensen and Schjoerring, 2001).

Do indicators of soil fertility and N availability predict variation in BNF across farms?

Given the critical role of legume biomass in determining the N supply from BNF, as well as the relevance of biomass to farm management, it is useful to understand drivers of variation in biomass across environmental conditions and management regimes. Legume biomass was higher in soils with lower N content in endogenous SOM pools, and increased with plant-available P concentration. POM pools are sensitive to management, and reflect differences in both the quantity and quality of organic matter inputs (Wander, 2004). The regression models were particularly strong for WP ($R^2=0.76$) and CC ($R^2=0.54-0.67$) biomass, which were negatively correlated with the amount of oPOM N, and with the total quantity of fPOM, both of which reflect N availability from mineralization from SOM. One legume, CV, showed an unexpected positive correlation with the fPOM pool, and, on average, the BNF rate and input for CV were much lower than for the overwintering legumes.

The models for BNF (shoot N from fixation; kg N ha^{-1}) corresponded with the results for legume biomass, though the fits tended to be weaker. These models indicated that legumes fix more N with increasing soil P concentrations, and, conversely, the % of legume shoot N from the soil was higher at lower soil P (i.e., when the % N from fixation was lower). Although WP and CC biomass were positively related to silt+clay content, contrary to the hypothesis, the model for

BNF (rather than biomass) indicated that CC BNF was negatively correlated with % clay. The poorer fit for models of RC in CC+RC+SW may be partly due to the low biomass production by RC, which tended to be outcompeted by CC and SW. However, Schipanski and Drinkwater (2011) did not find an inverse correlation between soil N availability and RC BNF across a farm gradient even with high RC biomass. In this study, the RC biomass in the RC+SW treatment was positively related to the C:N of the fPOM pool ($P < 0.0001$), indicating more biomass with lower quality POM.

Although fPOM pools tend to be larger on farms with a history of organic management (Wander et al., 1994, Marriott and Wander, 2006), the fPOM pool is also more ephemeral than oPOM and changes relatively quickly in response to organic matter inputs. The oPOM fraction turns over more slowly, and tends to be a more reliable indicator of longer-term changes in fertility due to management—making it possible to differentiate whether SOM stocks reflect background soil type versus management practices (Wander et al., 1994). I therefore expected to find stronger relationships with oPOM N, which was the case for CC and WP biomass. These findings contribute to ecological understanding of BNF within agroecosystems, and could inform development of management recommendations for farmers that provide estimates of BNF from mixtures to improve ecological N management.

Implications for agroecosystem management

Data from this study suggest that cover crop mixtures designed with complementary plant traits could increase the multifunctionality of agroecosystems. However, there were trade-offs among functions in which increasing functional diversity enhanced some functions and decreased others. These findings highlight the need to better understand competitive interactions in mixtures as well as feedbacks with soil properties, since variation in species performance across farms affects trait expression and associated functions.

Linking soil characteristics to mixture performance could inform adjustments to cover crop seeding rates in different conditions. For instance, grasses and brassicas in the mixtures tested here were more competitive with increasing soil fertility; their biomass increased with both fPOM pool size and P availability. Since P was also limiting to legume biomass across farms (i.e., there was a positive relationship between P and legume biomass), farms in the early stages of ecological nutrient management may require supplemental P additions or a greater proportion

of legume seeds within mixtures to increase the N supply from BNF. Legume biomass was strongly correlated with the N supply function across farms (Figure 3). Tools for farmers to predict biomass, along with models predicting mixture composition in different environmental conditions, could improve management recommendations based on functional ecology and ecological nutrient management frameworks.

Over time, regular use of legume N sources can increase labile soil N pools (Drinkwater et al., 1998, Schipanski and Drinkwater, 2011). Here, I found that the inverse relationship between soil N availability and BNF reported in more highly controlled conditions, often using synthetic N fertilizer, is also present on farms with organic nutrient management. These feedbacks would decrease BNF inputs at higher levels of N availability from SOM turnover, which corresponds with findings from on-farm research showing that legume N sources increase field-scale N use efficiency (Blesh and Drinkwater, 2013). Understanding how cover crop mixtures with complementary functional traits impact SOM pools over time could therefore inform adaptive management as soil properties change—improving management recommendations for farmers (e.g., selection of plant traits and appropriate mixture seeding rates) and reducing N surpluses that drive losses to surrounding ecosystems (Zhang et al., 2015). Research results from on-farm experimentation reflect realistic environmental and social contexts, and therefore have direct relevance to developing management systems that address critical sustainability goals.

ACKNOWLEDGEMENTS

The author would like to sincerely thank the farmer partners in this study, and Beth VanDusen, Eliot Jackson, Beth Dorgay, Rebecca Minardi, Alec Reznich, Andrew Harmon, Elliott Nichols, and Ryan Nelson for field and laboratory assistance. The author also wishes to thank Daniel Brainard and Sieglinde Snapp for input on the study design; the many volunteers who made it possible to sample all farm sites in a tight sampling window; and two editors at *Journal of Applied Ecology* and two anonymous reviewers for constructive comments that greatly improved the manuscript. This research was supported by a Ceres Trust Organic Research Initiative grant to J.B. and by the School for Environment and Sustainability at the University of Michigan.

DATA ACCESSIBILITY

All data used in this article are available on Deep Blue Data, the University of Michigan's digital repository for research data. DOI:10.7302/Z2WM1BK6 (Blesh, 2017).

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SUPPORTING INFORMATION

Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Cover crop treatment selection.

Appendix S2. Methods for POM fractionation and *B*-value determination.

Appendix S3. Functional diversity and ecosystem function calculations.

Table S1. Cover crop treatment details.

Table S2. Baseline soil properties at each farm site.

Figure S1. Aboveground biomass and multifunctionality.

Table 1. Multifunctionality assessment considering three ecosystem functions: N supply from BNF, weed suppression, and N retention in aboveground plant biomass. Maximum levels for each function across all treatments (determined by taking the mean of the top 10 observations across sites), and three different threshold levels (i.e., 30, 50, or 70% of the maximum level).

Ecosystem function	Maximum level	Threshold		
		30%	50%	75%
N supply from BNF (kg N ha ⁻¹)	154	46	77	115
Weed suppression (kg dm ha ⁻¹)	5683	1705	2842	4262
N retained in biomass (kg N ha ⁻¹)	196	59	98	147

Table 2. Mean (\pm standard error) Rao's Quadratic Entropy (Rao) and multifunctionality index value by treatment. Values labeled with the same letter were not significantly different at $P < 0.05\%$ (Tukey's HSD). Treatments with the largest index for each multifunctionality threshold level are in bold font.

Cover crop	Rao		MF 30%		MF 50%		MF 75%	
CC+RC+SW	1.2 \pm 0.1	c	2.5 \pm 0.1	f	1.6 \pm 0.2	cd	0.5 \pm 0.1	b
WP+OA+DR	1.4 \pm 0.1	c	2.3 \pm 0.1	ef	1.0 \pm 0.2	abc	0.3 \pm 0.1	ab
LN+YM+OA	0.5 \pm 0.1	b	1.7 \pm 0.1	bc	0.9 \pm 0.1	ab	0.3 \pm 0.1	ab
RC+SW	1.1 \pm 0.1	c	1.9 \pm 0.2	cde	1.0 \pm 0.2	abc	0	a
CC+SW	1.5 \pm 0.1	c	2.4 \pm 0.1	f	1.6 \pm 0.2	d	0.4 \pm 0.1	ab
CV+CR	2.1 \pm 0.2	d	2.0 \pm 0.1	cde	1.3 \pm 0.1	bd	0.2 \pm 0.1	ab
WP	0	a	2.2 \pm 0.1	df	0.9 \pm 0.2	ab	0.3 \pm 0.1	ab
CR	0	a	1.8 \pm 0.1	cd	1.5 \pm 0.1	cd	0.4 \pm 0.1	b
SW	0	a	1.4 \pm 0.1	ab	0.7 \pm 0.1	a	0.1 \pm 0.1	ab

Control (weeds)

-

| 1.0 ± 0.1 a

| 0.8 ± 0.1 a

| 0.3 ± 0.1 ab

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Table 3. Regression coefficients and standard errors (in parentheses) for regression analysis of legume biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R² and adjusted R².

LEGUMES

Treatment	CC+RC+SW	CC+SW	OA+WP+DR	WP	Treatment	CC+RC+SW	RC+SW	CV+CR
Species	CC	CC	WP	WP	Species	RC	RC	CV
Intercept	7089 (1783)	9391 (1787)	-1593 (1706)	2853 (1026)	Intercept	-405 (708)	-4029 (1023)	-25.9 (928)
Silt + Clay (%)	83.7** (27.9)	90.7** (28.0)	93.4*** (24.0)	60.9*** (16.1)	Clay (%)	9.5 (17.3)	16.4 (25.1)	-2 (22.8)
oPOM N	-4808*** (1117)	-5978*** (1119)	-2670*** (960.0)	-2008** (642.6)	C:N fPOM	26.5 (34.1)	255.9*** (49)	-1.9 (44.6)
fPOM pool	-98.8 (51.4)	-138.8* (51.5)	-165.2** (44.2)	-111.9*** (29.6)	fPOM pool	24.1 (16)	12.6 (23)	78.4*** (21)
Bray-1 P	12.5 (10.7)	18.4 (11.8)	73.9*** (9.2)	47.5*** (6.2)	Bray-1 P	0.13 (4.4)	3.5 (6.3)	-2.4 (5.7)

R ²	0.60	0.71	0.79	0.79	R ²	0.13	0.57	0.40
Adjusted R ²	0.54	0.67	0.76	0.76	Adjusted R ²	0	0.51	0.31
N	32	32	32	32	N	32	32	31

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

CC=Crimson clover; WP=Winter pea; RC=Red clover; CV=Chickling vetch

Table 4. Regression coefficients and standard errors (in parentheses) for regression analysis of non-legume biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R² and adjusted R².

NON-LEGUMES

Treatment	CC+RC+SW	CC+SW	RC+SW	SW	LN+YM+OA	LN+YM+OA	OA+WP+DR	OA+WP+DR	CV+CR	CR
Species	SW	SW	SW	SW	YM	OA	OA	DR	CR	CR
Intercept	-158.2 (932)	590.4 (1005)	-842 (965)	160.3 (1438)	-197 (1267)	-236 (455)	269 (202)	153.3 (553)	135.9 (2071)	2240 (2469)
Clay (%)	-20.2 (22.9)	-24.9 (25)	-54.9* (23.6)	-38.1 (35.2)	41.1 (31)	11 (11.1)	3.5 (4.9)	-20.9 (13.5)	232*** (51)	210** (60.5)

C:N free POM	21.1 (44.8)	-47.5 (48.4)	44.5 (46.4)	39.7 (69.2)	-28.4 (-61)	9.8 (21.8)	-11.2 (9.7)	6.8 (26.6)	-30.9 (99.7)	-143.1 (119)
fPOM pool	33.5 (20.9)	114.1*** (22.6)	93.1*** (21.7)	104.7** (32.3)	126.2*** (28)	-9.25 (10.2)	6.1 (4.5)	68.8*** (12.4)	-180.7*** 46.5	-13 (55.4)
Bray-1 P	12.5* (5.6)	4.5 (6.2)	25.4*** (5.9)	12.3 (8.9)	-0.2 (7.8)	7.6* (2.8)	-0.9 (1.2)	-5.9 (3.4)	70*** (12.8)	58.9*** (15.3)
R ²	0.29	0.58	0.67	0.37	0.54	0.25	0.2	0.57	0.64	0.44
Adjusted R ²	0.19	0.52	0.63	0.28	0.47	0.14	0.08	0.50	0.58	0.35
N	32	32	32	32	32	32	32	32	32	32

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

SW=Spring wheat; YM=Yellow mustard; OA=Oat; DR=Daikon radish; CR=Cereal rye

Table 5. Regression coefficients and standard errors (in parentheses) for regression analysis of weed biomass in each cover crop treatment using baseline soil properties as predictors. Coefficients in bold font are significant, and the estimated model fit is indicated by the R² and adjusted R².

WEEDS

Treatment	CC+RC+SW	CC+SW	RC+SW	SW	LN+YM+OA	OA+WP+DR	CV+CR	CR	Control
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Species	Weeds	Weeds	Weeds	Weeds	Weeds	Weeds	Weeds	Weeds	Weeds
Intercept	4646 (2241)	3630 (1615)	5169 (2423)	4730 (2319)	1857 (1299)	4339 (2254)	3315 (1807)	1572 (1539)	2516 (2140)
Clay (%)	96.5 (55)	87.4* (39.6)	189.2** (59.4)	220.2*** (57)	109.7** (31.8)	110.5 (55.2)	44.7 (44.3)	103.4* (37.7)	114.4* (52)
C:N free POM	-287* (107.8)	-254.2** (77.7)	-399.2** (116.6)	-329.4** (112)	-157.6* (62.5)	-284.9* (108)	-211.5* (87)	-171.9* (74)	-195.1 (103)
fPOM pool	27.5 (50.3)	39.4 (36.3)	20.2 (54.4)	-68.3 (52)	-3.9 (29.2)	34.34 (51)	103.2* (40.5)	22.3 (34.5)	134.1** (48)
Bray-1 P	13.8 (13.8)	18.5 (9.98)	29.7 (14.9)	18.6 (14.3)	22.5** (8)	24.7 (13.9)	8.4 (11.2)	14.7 (9.5)	37.7** (13)
R ²	0.3	0.44	0.45	0.41	0.41	0.34	0.44	0.36	0.53
Adjusted R ²	0.2	0.36	0.37	0.32	0.32	0.24	0.36	0.26	0.46
N	32	32	32	32	32	32	32	32	32

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

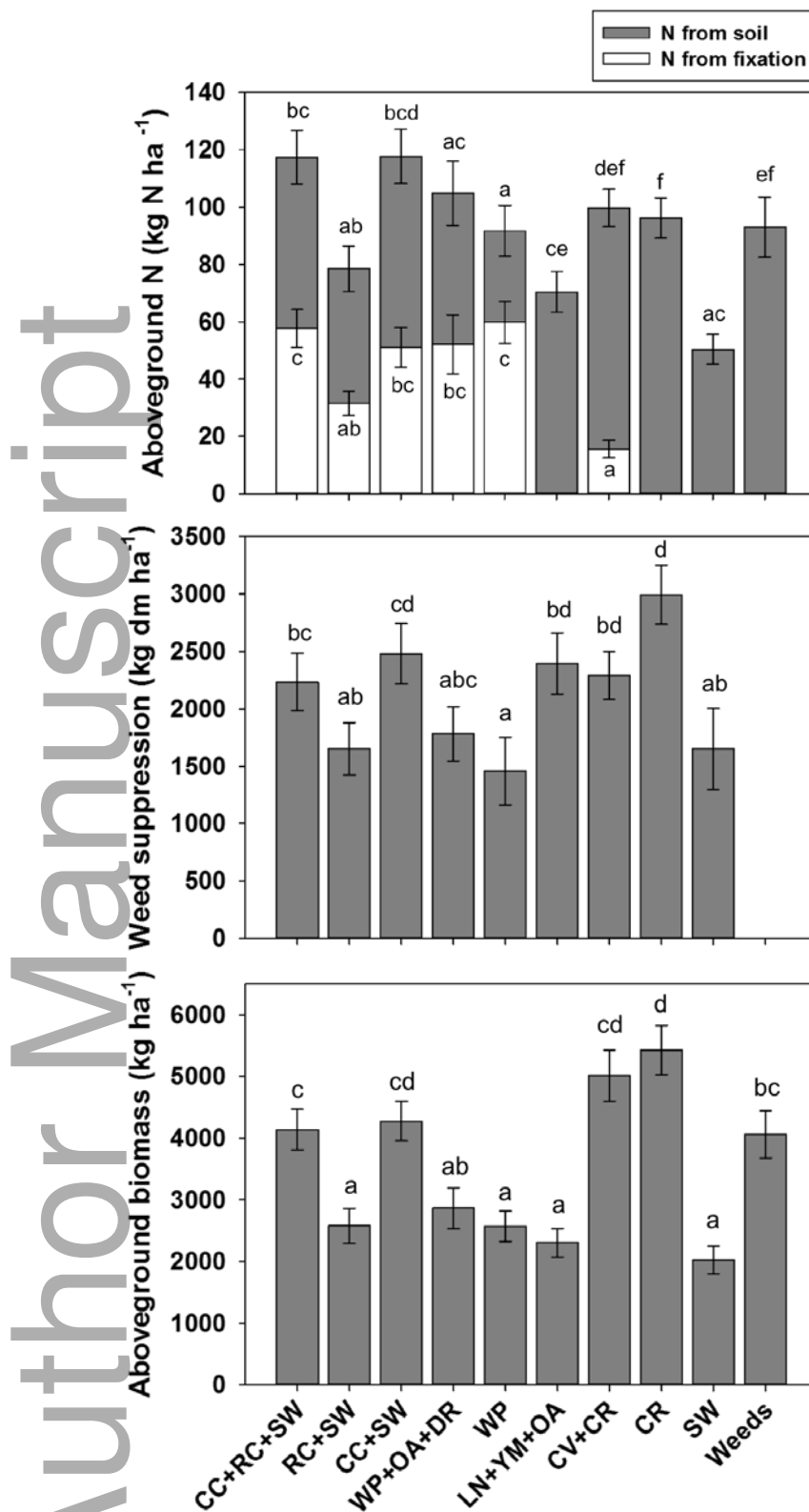


Figure 1. Treatment means and standard errors for ecosystem functions across farms for fall and spring combined. Top panel: N retention (gray portion of bar) and biological N fixation (white portion of bar); Middle: weed suppression; and Bottom: Aboveground biomass, combining species, and fall and spring sampling times. “Weeds” is the no cover control. Mean values labeled with the same letter were not significantly different at $P < 0.05\%$ (Tukey’s HSD). Abbreviations: CC= Crimson clover; RC= Red clover; SW= Spring wheat; WP= Austrian winter pea; OA= Oat; DR= Daikon radish; LN= Lentil; YM= Yellow mustard; CV= Chickling vetch; and CR= Cereal rye.

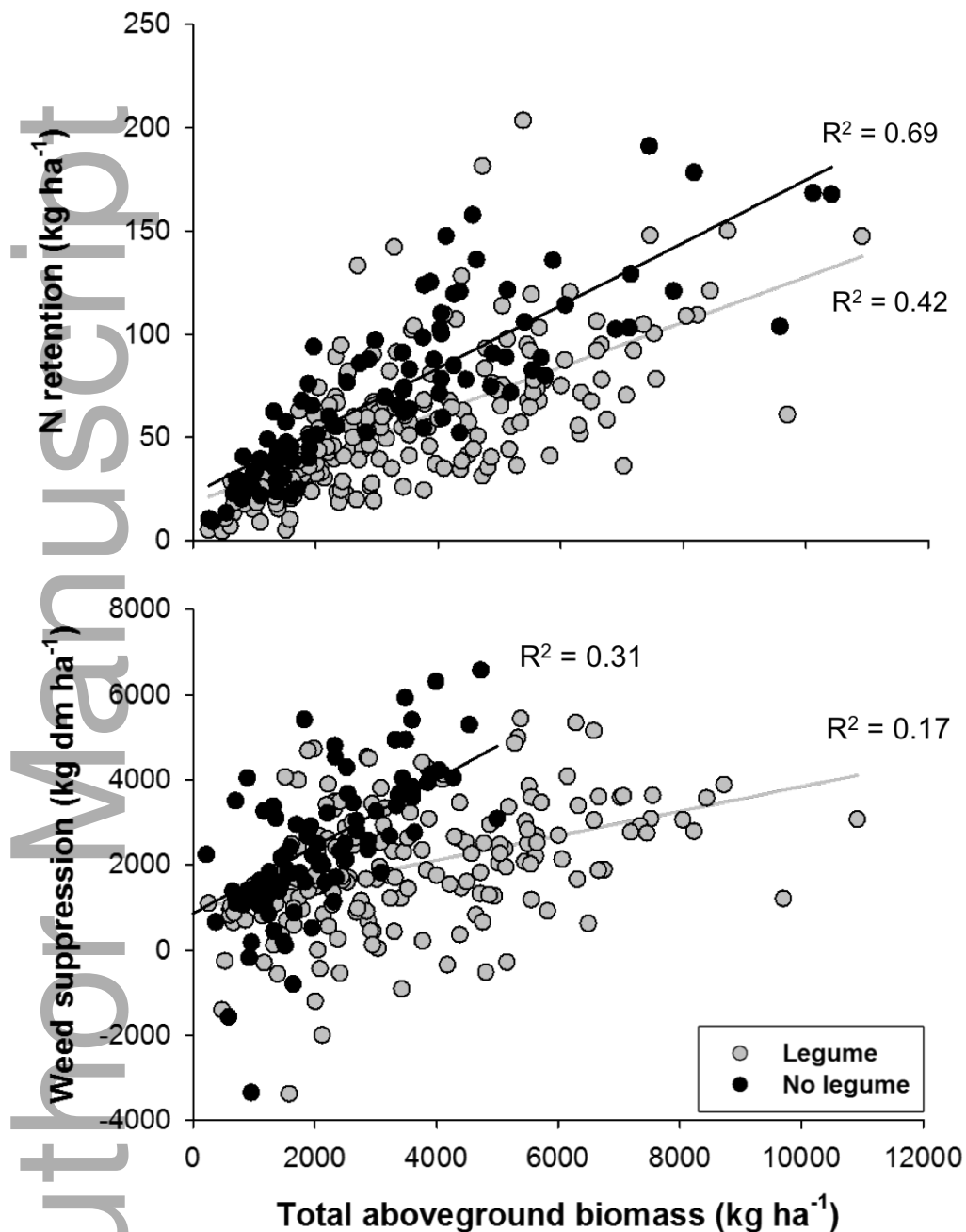


Figure 2. Regression relationships for total aboveground biomass (fall and spring sampling time points combined) and other ecosystem functions. Top: N retention (equal to total aboveground biomass N minus N from BNF), and Bottom: weed suppression (equal to weeds in control minus weeds in the treatment). Treatments are aggregated by those that include a legume species (gray symbols) and those that do not have a legume (black symbols).

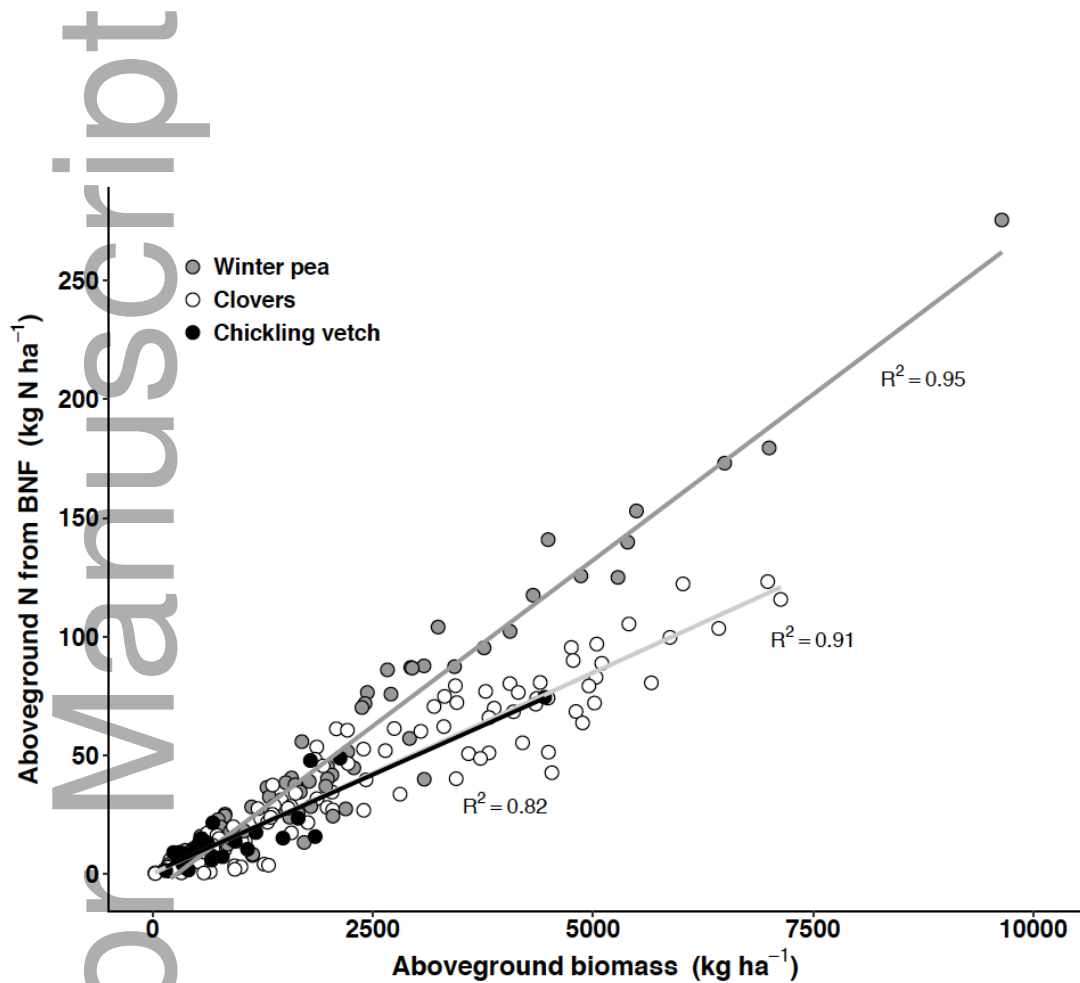


Figure 3. Relationships of legume aboveground biomass (combined fall and spring sampling points for winter pea and clovers) and the N supply function (total fixed N in aboveground biomass). Red clover and crimson clover are combined as “clovers”. Observations from all treatments were included in the analysis. Regression equations by species are: $y=0.028x-7.46$ (winter pea; $N=64$); $y=0.017x+0.70$ (chickling vetch; $N=26$); $y=0.018x-6.50$ (crimson clover; $N=64$); and $y=0.022x-0.84$ (red clover; $N=64$).

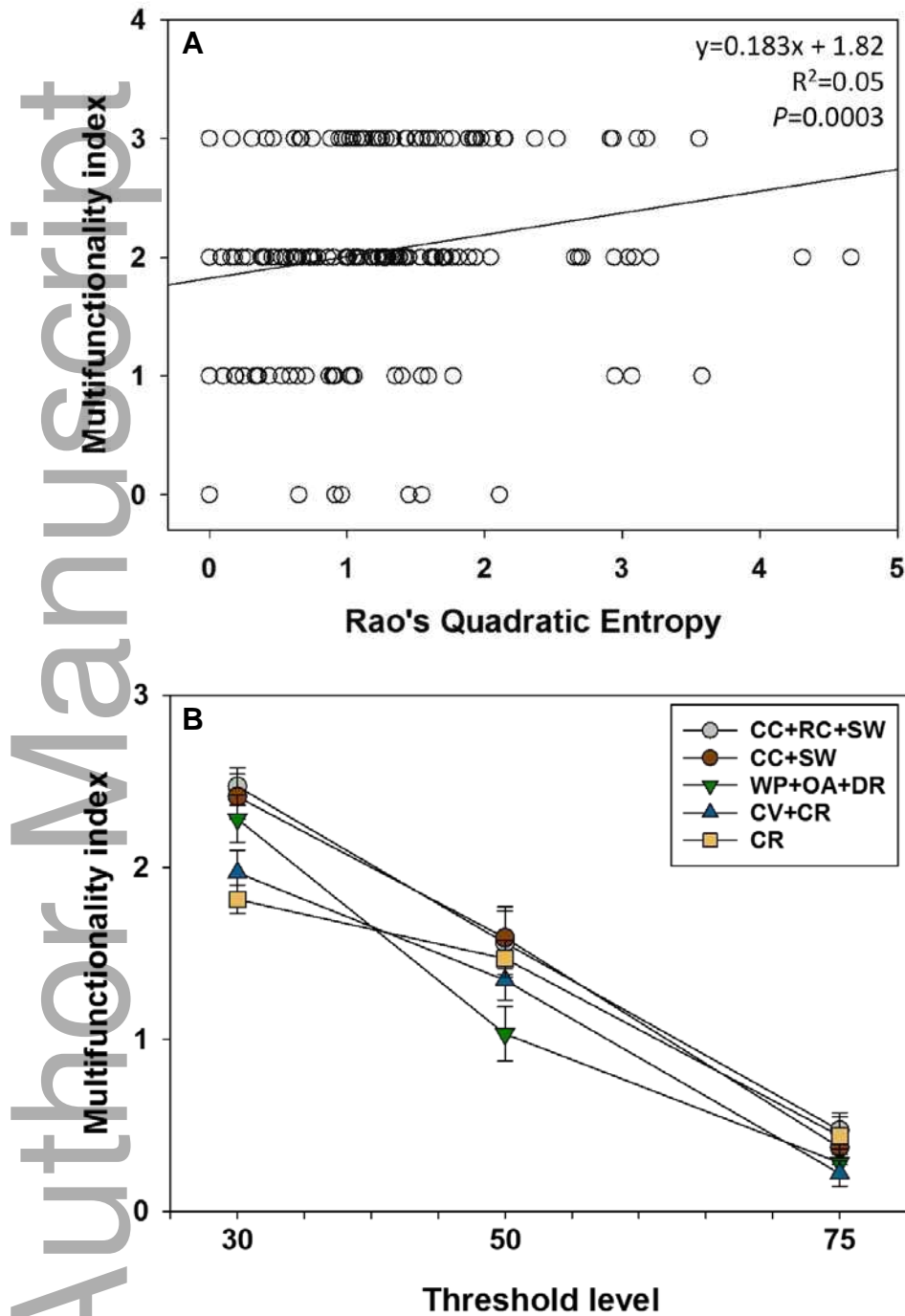


Figure 4. (A) Relationship between Rao's Quadratic Entropy (functional diversity) and multifunctionality for all cover crop treatments combined (for the 30% threshold level), and (B) mean multifunctionality index (with standard error) at the 30, 50, and 75% levels showing only the top five treatments at the 30% threshold level.